

B¹ All methods known in the prior art (von Eggeling and Spielvogel, 1995) have the disadvantage of insufficient sensitivity, however, because a relatively large number of cells must be used to increase the possibility of obtaining an amplification product. In addition, the sensitivity of the assay is reduced even more as the length of the fragment to be amplified increases. For this reason, the methods known in the prior art had only been used in the amplification of relatively small fragments with a length of up to 580 base pairs (Snabes et al., 1994).

✓ Please delete the paragraph at page 9, lines 3-4, and substitute therefor with the following paragraph: ✓

B² Figure 6A-6D: Mutation analysis of the P53 gene using DNA from breast carcinoma biopsies that was preamplified according to the invention.

✓ Please delete the paragraph at page 9, lines 6-13, and substitute therefor with the following paragraph: ✓

Figure 7: Detection of Ki-ras mutations on CK18-stained, disseminated tumor cells from bone marrow using RFLP. In each case, the DNA was applied in parallel undigested and digested with Mval.

B³ Lanes 1,2,: Immuncytochemically CK18-positive single cells from a patient with pancreatic cancer

Lanes 4,5: Immuncytochemically CK18-positive single cells from a patient with colon cancer

Lanes 3,6,7: A few immuncytochemically CK18-positive cells from a patient with colon cancer

Lanes 8-11: About 100 unstained, hematopoietic stem cells each as negative controls

24-29
✓ Please delete the paragraph at page 10, lines ~~6-13~~, and substitute therefor with the following paragraph: ✓

B⁴ Cell lysis was carried out according to the invention in 10 µl High Fidelity buffer (50 mM Tris-HCL, 22mM (NH₄)₂ SO₄ 2.5 mM MgCl₂, pH 8.9) which also contained 4 mg/ml

B4
cont.

proteinase K and 0.5 vol% Tween 20 (Merck) for 12 hours at 48°C. The enzyme was then inactivated for 15 minutes at 94°C. Lysis was performed in parallel batches according to the prior art (Zhang et al. 1992) in 5 µl 200 mM KOH, 50 mM dithiothreitol for 10 minutes at 65°. The batches were then neutralized with 5 µl 900 mM TrisHCl pH 8.3, 300 mM KCl.

✓
Please delete the paragraph beginning at page 18, line 22 and ending at page 19, line 3, and substitute therefor with the following paragraph:

B5

A comparison of the sequence analyses of DNA preamplified according to the invention with DNA from the same patient that was used directly in the sequencing revealed no difference in any of the patients investigated. The position and type of mutations identified are shown in Figure 6A-6D as an example. Figure 6A-6D shows a heterozygotic 8-base pair deletion in exon 7 (6A), a homozygotic C/G-G/C transversion (6B), a heterozygotic A/T-C/G transversion (6C), and a heterozygotic A/T-T/A transversion in exon 8 (6D) after primer-extension preamplification according to the invention. No further mutations were found. This means that preamplification according to the invention with an enzyme mixture of Taq polymerase and a polymerase with proofreading activity not only leads to increased sensitivity of amplification reactions and microsatellite analyses, but also that the amplification products exhibit no errors in their sequence and can therefore be used in mutation analysis.

✓ ✓
Please delete the paragraph at page 20, lines 16-29, and substitute therefor with the following paragraph:

B6

Colorectal carcinoma cells from the Lovo (ATCC) cell line were separated using a fluorescence-activated cell sorter. Poly (A) RNA was isolated from the individual cells using the Dynabead mRNA Direct Kit (Dynal, Hamburg). The entire batch was reverse-transcribed (20 µl according to the manufacturer) using SuperScript RNaseH reverse transcriptase (Life Technologies) and oligo (dT) primers. The resultant cDNA (20 µl) was used as a template for preamplification according to the invention in accordance with the preamplification PCR protocol described in Example 1. 1/30 of the cDNA preamplified in this fashion was then used as the template for amplification of a specific 408-bp fragment of